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Although the phenomenon of polyploidy has attracted the attention of research workers for approximately 25 years it was only recently that interest became general. Throughout the world investigations of an intensive nature are being conducted by many institutions in an attempt to determine the role which polyploidy has played in the evolution of species. This interest is attributed to the fact that about 50 per cent of the known species of higher plants, the Angiosperms, are polyploids, or of polyploid origin. In addition different workers succeeded in producing polyploid plants experimentally and the question arose as to whether those found in nature could have arisen in a manner similar to the methods employed in their experimental production.

It was soon realized that there were two kinds of polyploids, those involving duplication of the same genome and those involving a summation of different genomes. Kihara and Ono (1927) suggested the term autopolyploid for the former and allopolyploid for the latter. It is generally believed that allopolyploidy, particularly among the known species of higher plants, has played an important role in their evolutionary history. With respect to the evolutionary significance of autopolyploidy there exists a great diversity of opinion. In any case the whole question of polyploidy at present constitutes a special evolutionary problem of great importance.

As a result of the recent advances in the science of botanical phylogeny it has been possible to determine the probable ancestry of certain plant species. This has been accomplished through hybridization and a study of chromosome behaviour. In general it is assumed that modern descendants are in most instances derivatives of another or other species, usually members of the same genus. Clausen (1928) suggested that the origin of *Nicotiana tabacum* may be ascribed to doubling of the chromosome number in a hybrid between *N. sylvestris* and *N. tomentosa,* or close allies of these species followed by secondary alterations in factorial constitution and organization. Goodspeed and Clausen (1928) assumed that *tabacum* possessed two sets of chromosomes; one homologous with that of *sylvestris,* and the other with that of *tomentosa.* Brieger (1928a) reported a hybrid of *tabacum* with the 12-chromosome species, *N. Rusbyi* and (1928b) claimed that this hybrid exhibited *Drosera* type conjugation. It was shown by Kostoff (1930) that F_1 *sylvestris-Rusbyi* exhibited no conjugation of the chromosomes in meiosis, but that F_1 *tomentosa-Rusbyi* exhibited complete conjugation with only occasional irregularities. Goodspeed (1934) showed that F_1 *sylvestris-tomentosa* usually possessed two to three bivalents and may have as many as seven. The writer (unpublished data) has found that F_1*sylvestris-tomentosiformis* exhibits a comparable situation.

Goodspeed (1932) inquired into the taxonomic situation respecting *N. Rusbyi* and has decided to name it *N. tomentosiformis* owing to its pronounced resemblance and suggested close relationship to *N. tomentosa;* which fact had been established previously by Kostoff (1930). It was not surprising, therefore, to note that it exhibited parallel behaviour with *N. tomentosa* in crosses with *tabacum.* As suggested by Clausen (1932) these results indicate that *tomentosa*

and *tomentosiformis* are to be considered equivalent species in the above mentioned crosses. Throughout the remaining portion of the context of this bulletin "*N. Rusbyi*" will be referred to as "*N. tomentosiformis*".

Clausen (1932) inquired into the morphological differences between the F_1 hybrids of *sylvestris-tomentosiformis* and *sylvestris-tomentosa* and found that certain vegetative features as well as flower form, shape and colour of F_1 *sylvestris-tomentosiformis* displayed a greater resemblance to those of *tabacum* than those of F_1 *sylvestris-tomentosa.* It was decided, therefore, that *tomentosiformis* more nearly satisfies the requirements of a progenitor of *tabacum* than *tomentosa.* Cytological evidence presented by Goodspeed (1934) dealing with the amphidiploid origin of *N. tabacum* found that there was thorough justification to conclude that some progenitor of *tomentosa* or *tomentosiformis* along with some progenitor of modern *sylvestris* entered into the production through amphidiploidy of the highly polymorphic modern species called *tabacum.*

N. triplex

As suggested above, *N. tabacum* consist of two subgenomes, one of which is derived from *N. sylvestris* and the other from *N. tomentosiformis.* Brieger (1930) obtained a trigenomatic plant from hybridization of F_1 *tomentosiformis-tabacum* with *sylvestris.* A second was obtained by Kostoff (1931) from *tabacum* $\times$ F_1 *sylvestris-tomentosiformis.* Later, Kostoff (1933) reporting on the cytogenetic studies of the triple fertile hybrid *N. tabacum* $\times$ (*N. sylvestris N. Rusbyi*)—*N. triplex* suggested that the *N. tabacum* genome was not identical with the *sylvestris* + *tomentosa* genome. He also stated that this method of obtaining triple fertile hybrids should be of value to plant breeders in combining the characters of three different species into a fully fertile plant. The triple hybrid which constitutes the basis for this investigation was obtained by Clausen from hybridization of F_1 *tabacum-tomentosiformis* $\times$ *sylvestris.* The plants developed from this hybrid were highly fertile and it is assumed that they arose from unreduced gametes produced by the hybrid in question.

Since the trigenomatic plants produced in this way consist of 24 chromosomes from *N. tabacum* and 12 homologues from each of *sylvestris* and *tomentosiformis*, they are regarded as triple hybrids and consequently referred to as "*Nicotiana triplex*". These triple hybrids or trigenomatic *sylvestris-tomentosiformis-tabacum* plants exhibit 24_{II} chromosomes in meiosis, but due to structural differences in the organization of the chromosomes involved, or slight genic differences in the various homologues of some bivalents, at least, segregation of certain characters in the immediate progenies is to be expected.

Purpose of the Investigation

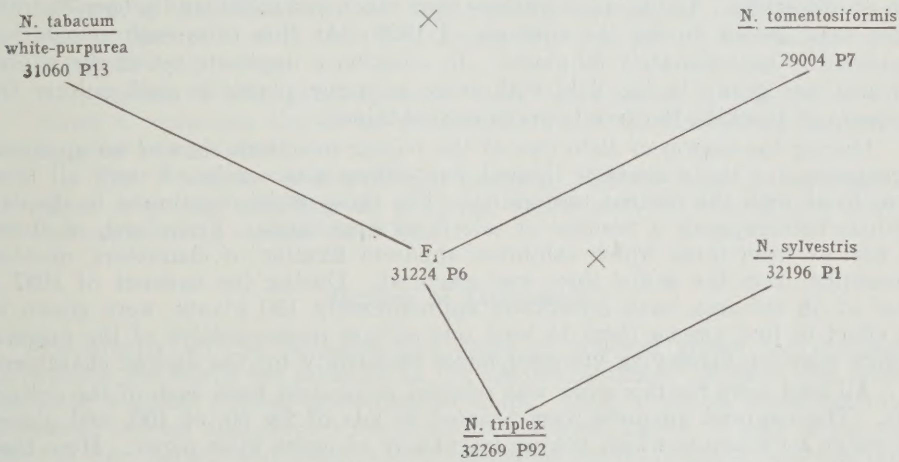
The writer's attention was drawn to the morphological differences exhibited by the derivatives of the trigenomatic plants obtained from F_1 *tabacum-tomentosiformis* $\times$ *sylvestris.* It was noticed that these trigenomatic plants were highly variable despite the fact that they came from unreduced gametes. This was due, no doubt, to crossing-over between *tomentosiformis* chromosomes and their *tabacum* homologues, as was evidenced by their conjugation. The diversity of these derivatives as well as the morphological differences between *tabacum* and F_1 *sylvestris-tomentosiformis* indicated extensive differences between *tabacum* chromosomes and their *tomentosiformis-sylvestris* homologues. Although these differences may be essentially genic in nature there is some evidence of minor structural ones as well.

It was felt therefore that certain segregants among the various derivatives should be selected in an attempt to establish a number of types by selfing over a period of two or three generations. Aside from the white flower colour which

may have been introduced by *tabacum* all the segregation products obtained following selection and selfing rest upon the introduction of elements from *sylvestris* and *tomentosiformis.* It is intended to show that numerous genetic differences may be introduced from these species into the *tabacum* complex by the method here employed. The present investigation, however, is designed to analyse on a genetical basis the various characters displayed by the trigenomatic segregation products and to determine their genetical relationship to existing *N. tabacum* types.

Materials and Methods

The various segregants, the characters of which are being analysed, are all derivatives of a *N. triplex* plant obtained by Dr. R. E. Clausen in his experimental plots at the University of California, Berkeley, California. The origin of this plant, designated 32269 P22, is illustrated below. By way of explanation, it may be suggested that this number is a composite one which could be referred to as 32-269-P22. The figure 32 refers to the year in which the culture was grown, that is, 1932; 269 refers to the plot or strain number; while P22 refers to the particular plant in this culture or population.



As is illustrated above, *N. tabacum,* 31060 P13 var. purpurea-white, was fertilized with pollen from *N. tomentosiformis* 29004 P7. From this cross was derived a hybrid 31224, which at meiosis revealed, in support of the evidence presented above, $12_{II}+12_{I}$ chromosomes. The plants in this population were very uniform in all respects. They were all highly sterile, but usually retained their capsules on pollination with *sylvestris* although the capsules each contained only a small number of seeds. An attempt was made to fertilize plant No. 6 (P6) of this population with pollen from *N. sylvestris* 32196 P1. From this cross a number of seeds were obtained. These when sown the following year produced about 150 plants which constitute the culture 32269. Among the plants in this population about one-third were trigenomatic and P92 was selected because of its superior fertility. This population was not studied carefully but there was great variability both in morphology and fertility among the trigenomatic plants. In this instance it can only be assumed, in the absence of further confirmatory evidence, that at least one unreduced gamete was produced by the F_1 hybrid 31224 P6, and that this gamete was fertilized by a normal one contributed by *N. sylvestris* 32196 P1. The result obtained from the immediate progeny of the last-mentioned cross was a number of highly fertile trigenomatic plants in the culture numbered 32269.

During the summer of 1933 a number of *tabacum* monosomics were crossed with pollen from 32269 P92. Progenies of these crosses were grown in 1934, and all were selfed at the end of the season. Selfed populations from each of these were set out in 1935, and at that time a number of selections were made. Each of the selections made displayed certain differences with respect to leaf shape, flower colour and habit of growth. Complete notes were taken of each selection in particular as well as all plants of the entire culture from which each of the individual selections was made. These notes included such data as number of leaves to the first bald sucker; height of plant; shape of leaf; length and width of middle leaf; and colour, size, and shape of flower. It might appear that since certain of these observations are given no consideration in the genetical analysis which follows, the notes taken are unnecessarily comprehensive. The extreme importance of this precaution is only recognized when it is realized that selections are made from segregating populations, and the only means of recovering the particular type desired in succeeding generations is by strict and constant reference to previous records pertaining to that type.

During the summer of 1935, six outstanding *triplex* types were selected and selfed. Twenty-five plants from each selection were grown in the greenhouse during the winter 1935-36. All cultures continued to show considerable segregation of characters. Additional selections were made and selfed and progenies from these were grown during the summer of 1936. At this time each population consisted of approximately 50 plants. In addition a duplicate set of the winter cultures was grown in the field with twice as many plants in each culture for purposes of checking the results previously obtained.

During the season of 1936 two of the *triplex* selections showed no apparent segregation for the characters desired but selfing was continued until all lines were fixed with the desired uniformity. For those which continued to display obvious heterozygosis a number of selections were made. From each of these, as well as from those which exhibited apparent fixation of characters, another generation from the selfed lines was essential. During the summer of 1937 a total of 58 cultures, each containing approximately 100 plants, were grown in an effort to find among them at least one culture representative of the original *triplex* selection displaying unquestionable uniformity for the desired characters.

All seed sown for this work was selected at random from each of the culture lots. The required amounts were counted in lots of 25, 50, or 100, and placed on water germinators which consist essentially of moist filter paper. Here they were allowed to germinate for a period of some 10 days, or until such time as it was deemed advisable to transfer the young seedlings to small 3″ pots. Approximately 30 days' growth was required in these pots before they were transplanted to flats. Another 25 to 30 days were usually required before the seedlings were of sufficient size for transferring from the flats to the field, or to large 3-gallon pots for greenhouse maintenance.

For the purpose of ensuring self-fertilized seed all pollinations were conducted by hand and each pollinated seed head was bagged to prevent any contamination which might be attributed to insects.

In the field or greenhouse each plant was numbered and notes of characters were recorded individually on blank forms. When necessary the cultures were examined several times during the season. All data recorded on segregating populations from which selection of types for selfing was being conducted were made from a detailed study of the plant in the field. Since the selfed *triplex* lines were established during the summer of 1937 these were hybridized with certain *tabacum* types and F_1 hybrids of all *triplex* types with *tabacum* were grown in the greenhouse during the winter of 1937-38. During the summer of 1938 parental *triplex* and *tabacum* types, as well as F_1 and F_2 *triplex-tabacum*

hybrids were all grown in the field according to a designed arrangement which facilitated careful classification. In the study of leaf characters records were made not only from examination but also from outline drawings made of a representative leaf picked from each plant for all cultures.

Since all the plants in each F_2 population were studied individually it was found that the segregants with respect to certain characters, if not of the same type, could be classified as belonging to one or other of two or more main groups. Each group contained all the individuals displaying a particular phenotypic expression. In attempting to account for the various phenotypes encountered in any population and the number of individuals in each phenotypic group, the genetic constitution of the parents and the progenies was postulated. With a series of observations of this kind it is necessary to compare the results with those expected on the basis of the hypothesis suggested for any particular case. This is referred to as a test of Goodness of Fit. Tests of this kind were applied to the results obtained from all F_2 populations derived from the crosses involving triplex selections and *tabacum* types. The method followed is that described by Fisher (1936) and Goulden (1937) where use is made of the known distribution of the χ^2 (chi-square). The formulae used were as follows:

(1) For simple ratios involving two classes: $\chi^2 = (a_1 - a_2 x)^2 / x N$ where a_1=no. of individuals in the larger group, a_2=no. of individuals in the smaller group, x=3 if ratio is 3:1 and N=total no. of individuals in the population.

(2) For ratios involving more than two classes: $\chi^2 = \Sigma\ (a - t)^2\ /\ t$ where a represents the actual frequencies and t the theoretical frequencies in terms of the latter.

The P values, 0·10-0·20 for instance, indicate that a deviation as great or greater than the one observed would occur in 10 to 20 per cent of the cases due to chance variation.

Review of Literature

Practically all the varieties and types of tobacco grown for commerce throughout the world are included in the species *tabacum*. Within this species are found a highly diverse assemblage of forms. Most of the varieties cultivated at present possess pink flowers and lanceolate shaped leaves. These leaves may differ in size—length and width, colour—different shades of green, and a number of other features of great economic importance such as feel, body, and texture. Other varieties display more marked morphological differences. A number of attempts have been made to determine the mode of origin of these varieties and to set up schemes for their classification. Comes (1905) and Anastasia (1906) claimed that all varieties had arisen from relatively few basic types through hybridization. The Howards (1910, also 1913) and Setchell, Goodspeed, Clausen (1922) have shown, however, that the morphological criteria which these authors employed are not necessarily indicative of genetic affinities, for a number of similar morphological characters have been found to differ in genetic constitution.

Kelaney (1925) attributed certain leaf-base types to the segregation of two pairs of factors. The genetic constitutions S S A A, S S a a, s s A A, S s A a, and s s a a conditioned the expression of five leaf-base characters, lanceolate, petioled, broad, short-petioled F_1 and constricted, respectively. In addition, two pairs of factors accounted for the relation existing between red, light pink, and white flower colours. A third pair of factors was necessary to account for carmine. A linkage with 7·5 per cent crossing-over was found to exist between the factors responsible for broad leaf-base and pink flower

colour. Bourzev (1928) distinguished five leaf-base forms, petioled, winged petioled, subpetioled, sessile leaf with narrow base, and sessile leaf with broad base. A preliminary attempt was made to account for most of the forms on a trihybrid basis—a few types being heterozygous for certain factors.

Gentscheff (1935/36) reported on the results obtained from crosses between certain sessile varieties (babura, alba, and macrophylla) and petioled varieties (sanguinea and angustifolia) and concluded that three pairs of factors were involved. Babura, alba and macrophylla could be represented by the following genotypes X X Y Y Z Z, X X Y Y z z and x x y y Z Z whereas sanguinea and angustifolia were x x y y z z.

The segregation of leaf colours, yellow green and dark green has been reported by Henika (1932) and Christoff and Gentscheff (1935). The expression of the yellow green character was found to be conditioned by the presence of two pairs of recessive factors.

Gentscheff's work (1935) on corolla colour and shape confirms the results obtained by Setchell, Goodspeed and Clausen (1922) with respect to the inheritance of flower colour and claims that flower shape may be explained on a bifactorial basis with intermediate forms in the heterozygous condition.

Leaf Base Types

The *N. triplex* selections obtained as a result of continued selfing differ in leaf-base types. An attempt was made to develop types comparable in appearance to those previously subjected to investigation and analysis at the University of California. In addition, a particular effort was made to establish strains not included in the earlier work so that all may be incorporated into these investigations. With respect to this feature it was possible to distinguish and establish six main forms which are designated: broad, semi-broad, lanceolate, petiolate, winged petiolate and constricted. These may be described as follows:—

Constricted.—Plate I, fig. 1 and Plate V, fig. 2 illustrate the constricted type. Plate I, fig. 1 represents a typical leaf of 37072. Here the petiole is very short with narrow wings sharply constricted. The blade is obliquely obtuse to rounded and auricles broadly lunate and decurrent on the stem.

Lanceolate.—Lanceolate leaves are essentially sessile. The blade is sub-cuneate at the base and continuous with broadly rounded auricles decurrent on the stem. As shown in Plate I, fig. 2, a typical leaf of 37288, the leaves are longer and narrower than those of the broad type.

Broad.—Leaves are definitely sessile with a comparatively wide blade which is broadly cuneate and continuous with auricles. The auricles are broadly deltoid to rounded as shown in Plate II.

Semi-broad.—These types appeared to be intermediate broad which at first seemed impossible of fixation. They possess a short petiole with broad wings. The blade is sub-cuneate at the base, and auricles lanceolate and decurrent. *See* Plate III.

Petiolate.—In this type the leaves possess a distinct petiole which is somewhat variable in length. The blade of the long petiolate form is sub-cuneate to cuneate at the base with auricles setaceous to linear (*see* Plate IV) and decurrent on the stem. The shorter petiolate form usually possesses a blade which is obtuse to rounded at the base and slightly oblique. As shown in figure 2 the auricles are narrowly lunate and decurrent on the stem.

Long-winged-petiolate.—Although this type, as illustrated in Plate V, fig. 1, comes under the general classification of petiolate it possesses definitely smooth wings. The blade is cuneate at the base and the auricles narrowly lanceolate, decurrent on the stem.

FIG. 1

FIG. 2

PLATE II

FIG. 2

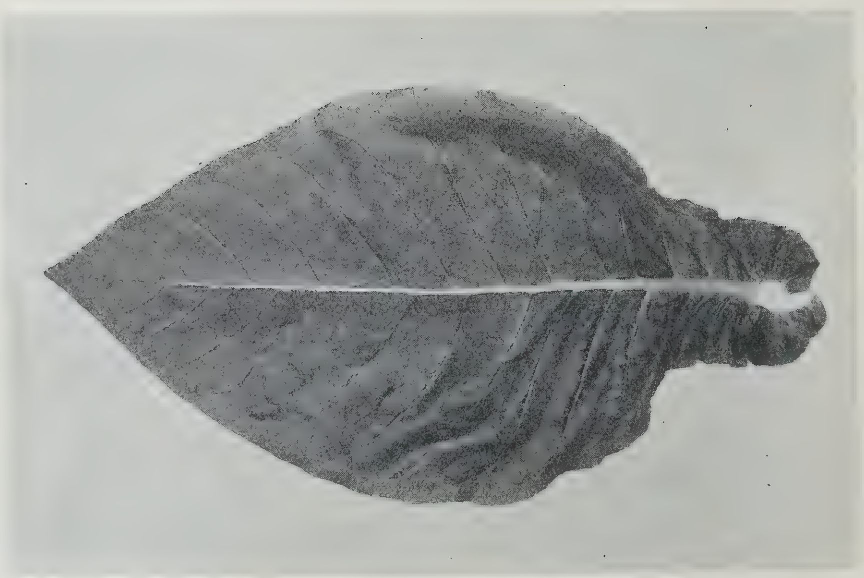


FIG. 1

PLATE III

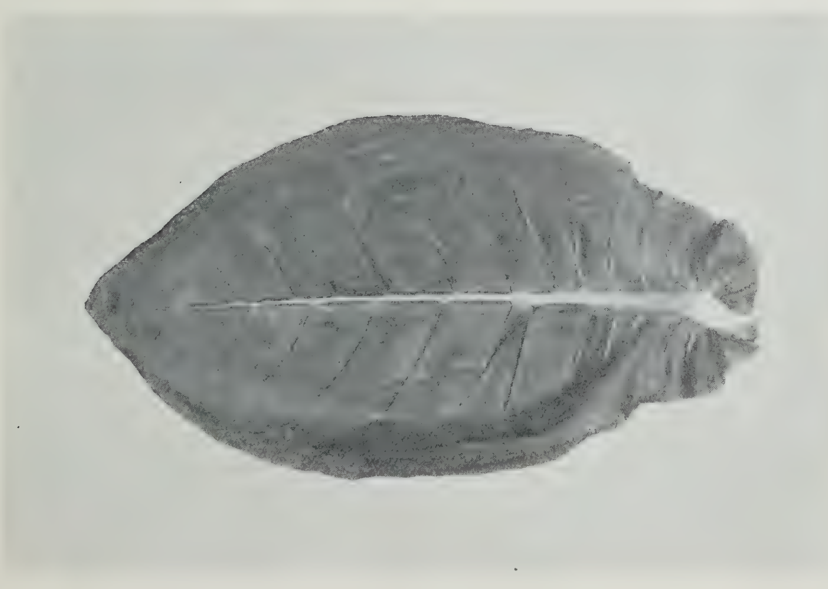


FIG. 1

FIG. 2

PLATE IV

FIG. 1

FIG. 2

PLATE V

FIG. 1

FIG. 2

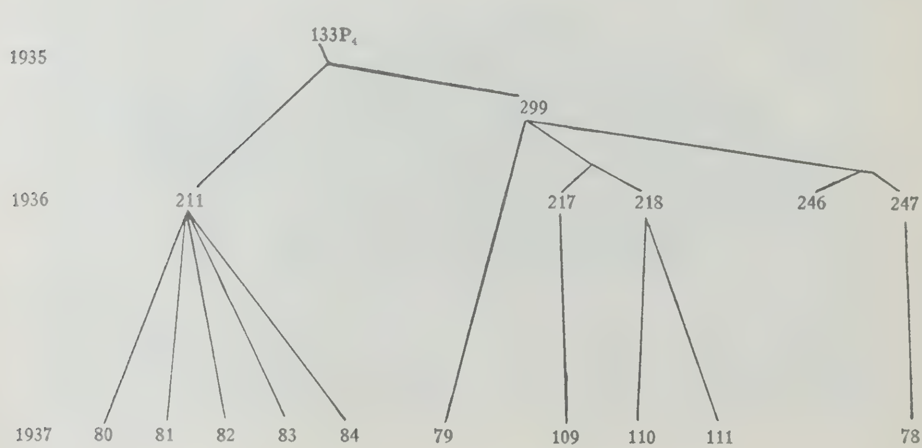
Initial selections were made from a number of *N. triplex* cultures in Dr. Clausen's experimental plots at the University of California during the summer of 1935. Culture numbers from which selections were made included the following: 35133, 35135, 35139, 35149, 35155 and 35157. All of these displayed definite segregation for leaf shape and flower colour. As for the leaf base types described, which were derivatives of the original trigenomatic plant, it is to be noted that constricted is the one appropriate to the *tabacum* parent. The rest must have been segregation products incorporating *sylvestris* or *tomentosiformis* elements. Since each of these cultures as well as those of the first two generations contained only a limited number of plants no attempt was made to determine linkage or cross-over values throughout the selfed lines.

Selfed Lines

Culture number 35133 consisted of 25 plants, 6 of which possessed broad leaf bases, 15 semi-broad and 4 constricted. The flower colour ranged from carmine to white, 8 carmine, 11 dull carmine, 4 pink, and 2 white. The selection P_4 from this population possessed a semi-broad leaf base and a dull carmine flower colour. The next generation 35299 produced plants with all semi-broad leaves but segregated for flower colour, 2 carmine, 13 carmine dull, 7 pink and three white. The progeny 36211 from 35133P_4 semi-broad and carmine dull consisted of 35 plants, of which 19 were carmine or dull carmine, 12 pink and 4 white. Similar results, as shown below, were obtained from cultures numbered 36246 and 36247.

On examining the culture 35299 it was felt that probably the dull carmine flower colour was attributable to a heterozygous condition which could not be established. Additional selections therefore were made for normal pink and white flower colours.

The cultures obtained from selection 35133P_4 are presented below.



As previously intimated all progenies from selections possessing dull carmine coloured flowers segregated for carmine, pink, and white. The results obtained from these cultures are contained in table 1.

TABLE 1

Segregation for flower colour from dull carmine selections.

Garden Number	Carmine and Carmine Dull	Pink	White
35299	15	7	3
36211	19	12	4
36246	29	8	2
36247	33	14	7
Total	96	41	16
Calculated 9 : 3 : 4	86·0	28·7	38·3

It would appear, therefore, that dull carmine flower colour was a character conditioned by a heterozygous genotype. This condition can be explained on the assumption that carmine flower colour may be attributed to the presence of two pairs of factors. Using the factor notation adopted by Clausen (1932) the carmine set up could be represented by WhWhPkPk where the symbols Wh-wh are used for coloured vs. white and Pk-pk for carmine vs. pink. According to this scheme the various phenotypic classes should be:—

WhWhPkPk—carmine.
WhWhpkpk—pink.
whwhPkPk—white.
whwhpkpk—white.

Although the results presented in table 1 are not indicative of a 9:3:4 ratio it is felt that greater accuracy would have been obtained if larger populations were grown for each of the cultures involved. Confirmation of the assumption, however, is to be found in a study of other segregants from the original selection and segregants from other selections discussed below.

Cultures numbered 36217, 36218, and 37082 segregated for pink and white flower colour as indicated in table 2.

TABLE 2

Segregation for flower colour from pink flower colour selections.

Garden Number	Pink	White
36217	19	8
36218	25	6
37082	71	22
37110	69	19
37111	55	26
Total	239	81
Calculated 3 : 1	240	80

The results obtained in the above table suggest that pink and white flower colours are expressions of the same pair of factors. The dominant factors account for the pink coloured flower, whereas the white flower is attributed to

a single pair of recessive factors. As carmine flower colours were not present it is assumed that the pink colour may be represented by WhWhpkpk or Whwhpkpk. Since the pink flower colour selections segregated for pink and white in a definite 3:1 ratio the pink selection must have been a heterozygous genotype and the white segregants of a whwhpkpk genetic constitution. In this instance, however, it is only necessary to assume the existence of a single pair of factors of a heterozygous genotype.

Certain carmine flower coloured selections segregated for carmine and pink alone. The results from these selections are given in table 3.

TABLE 3

Segregation for flower colour from carmine flower colour selections.

Garden Number	Carmine	Pink
37080	41	14
37083	58	18
37084	72	26
Total	171	58
Calculated 3 : 1	173·2	55·8

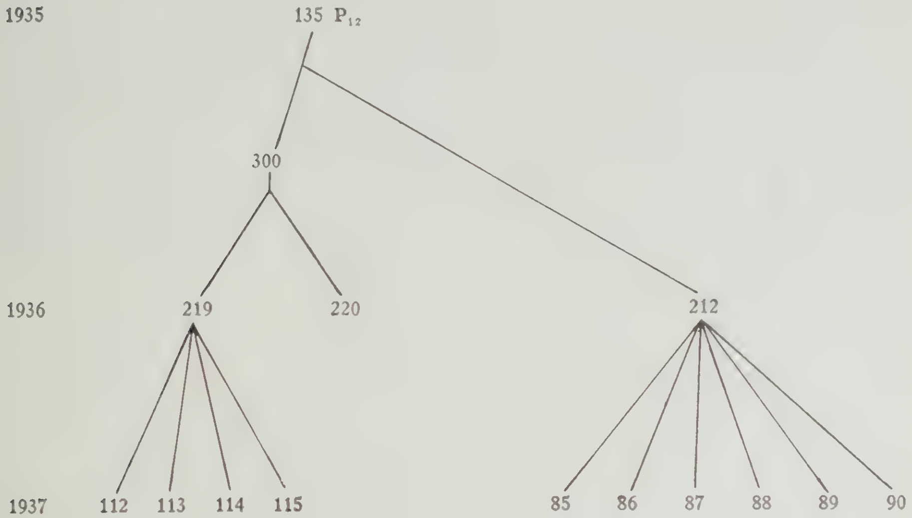
The data presented in table 1 have demonstrated that the various segregants for carmine, pink and white could be accounted for on the basis of two pairs of factors. Mindful of the results presented in tables 1 and 2 it is also possible to account for the results in table 3 in agreement with the two factor hypothesis if we assume that the genetic constitution of the carmine flower colour may be represented by WhWhPkPk, WhwhPkPk, WhWhPkpk or WhwhPkpk and pink by WhWhpkpk or Whwhpkpk. Since, however, no white flowered segregants appeared in either of the populations referred to in table 3 it is only necessary to assume that a single pair of factors were involved and that carmine flowers were either PkPk or Pkpk and the pink ones were pkpk.

All plants in cultures numbered 37078 and 37081 were white and may be represented by the genetic constitution whwhPkPk or whwhpkpk whereas all plants in culture numbered 37109 were pink and may be represented by the genetic constitution WhWhpkpk. In addition, all segregants from the original selection 35133 P_4 possessed leaves of the semi-broad type. This character will be dealt with later.

35135

In culture numbered 35135 there were 12 carmine coloured flowers, 7 pink and 6 white. The leaf shapes segregated for broad and constricted, 17 and 8 respectively. Selection P_{12} from this population possessed a very broad leaf base with pink flowers. The progeny 35300 from this selection was grown in the greenhouse the following winter and appeared to show slight variation in type of leaf base which was difficult to classify. Growing conditions in the greenhouse undoubtedly contributed in some measure to this difficulty. A portion of the population obtained from selfed seed from 35300 P_{16} consisted of plants with winged petiole. Since this character was not encountered in any of the original cultures from which the first selections were made it was deemed advisable to select a typical winged petiolate type.

The cultures obtained from 35135 P_{12} are presented below:—



As stated above a winged petiolate type of leaf was encountered in culture numbered 36219. A selection from this population 36219 P_6 resulted in a winged petiolate population the following year, 37112. All the other selections from broad leaf base types produced cultures displaying only broad leaf base types. With respect to flower colour, cultures numbered 37112, 37114, 37115, 37085 and 37090 produced all pink flowers from pink selections, whereas 37087 contained all white flowers produced from white flowered selections. The remaining cultures all segregated for pink and white from, presumably, heterozygous pink selections.

TABLE 4

Segregation for flower colour from heterozygous pink selections.

Garden Number	Pink	White
35300	21	2
36219	19	12
36220	23	12
36212	18	6
37113	40	15
37086	44	13
37088	56	16
37089	59	14
Total	280	90
Calculated 3 : 1	277·5	92·5

The above results compare favourably with those presented in table 2. The three to one ratio obtained in this instance confirms the assumption that both colours may be explained on a monohybrid basis with the genetic constitutions WhWh, Whwh, and whwh accounting for pure pink, heterozygous pink and white respectively.

35139

An attempt was made to select from culture numbered 35139 a plant with a leaf shape which resembled that of normal *N. tomentosiformis*. The selection P_7 made from this population was designated Br- to denote a leaf base shape which was neither definitely broad nor constricted, the characters for which the culture appeared to be segregating. Recognizing only the existence of these characters in the classification of this culture of 25 plants a ratio of 6 broad: 10 semi-broad: 9 constricted was obtained. With respect to flower colour only 24 plants were analysed as one failed to mature, the result being, 15 carmine, 3 carmine with white throats and pale filaments and 6 pink, or, if all carmine coloured flowers are considered together, a perfect 3:1 ratio is obtained in 18 carmine to 6 pink.

The cultures obtained from 35139 P_7 are presented below.

As no particular purpose will be served by giving the full details of the classification resulting from an analysis of all cultures developed from the above selection, only those of the first and second generation segregating for flower colour and leaf shape will be considered. Additional tables would constitute unnecessary repetition.

TABLE 5

Segregation for flower colour and leaf shape.

Garden Number	Flower Colour		Leaf Shape	
	Carmine	Pink	Broad	Constricted
35139	18	6	16	9
35301	21	4	17	8
36213	16	9	22	3
36221	26	8	23	11
36222	35	7	33	9
Total	116	34	111	40
Calculated 3 : 1	115	35	113·2	37·8

With respect to flower colour the figures presented in table 5 are in agreement with those presented in tables 1 and 3. As for shapes of leaf bases the results here obtained are in accordance with those reported by Kelaney (1925). For the purpose of showing the relationship between flower colour and leaf base segregation the totals as presented in the preceding table are classified below:—

Ratio	Phenotypes	Observed	Calculated
1	Broad pink	34	37·5
2	Broad carmine	76	75
1	Constricted carmine	40	37·5
	$\chi^2=0\cdot51$ P=0·7—0·8		

Since there were no constricted pink individuals in any of the cultures referred to in table 5 it was assumed that the factors conditioning leaf shape were closely linked with those responsible for flower colour. In order to account for the various classes observed it must also be assumed that the broad (Br) and pink (pk) factors are members of one linkage group and that constricted (br) and carmine (Pk) are factors which are contained in its homologue.

In view of the fact that two winged petiolate populations 37094 and 37117 were developed from the original selection 35139P_7, it does not appear that this type should necessarily be regarded as an expression of a heterozygous condition of a single pair of factors. At the same time its occurrence here and in the previous pedigree as a derivative from 35135 makes it exceedingly difficult to explain on a Mendelian basis, unless it represents another instance of like phenotypes with a different genetic basis. For further investigation, however, specimen 37094 P_{21} with winged petiole and carmine white throat and pale filament flowers was selected.

35149

From this culture a constricted leaf base type with carmine flower colour, white throat with pale filaments was selected. No difficulty was encountered in fixing the type although there appeared among the progenies of the early generations certain minor differences in leaf shape which could only be attributed to the presence of slight modifying factors. These differences were not sufficiently marked to allow for detailed classification. Consequently all plants in each of the cultures were grouped under the general classification of "constricted." The white throat and filament colour character reproduced itself in the various generations as a simple recessive.

35157

Culture number 35157 segregated for pink and white flower, 18 and 5 respectively and broad and constricted leaf bases, 15 and 8 respectively. P_{21} of this culture possessed a very broad leaf base and the second generation 35304 from this selection gave all broad leaves and pink flowers except one plant which possessed a distinctly petiolate leaf. All progenies from selfed selections with broad bases and pink flowers gave consistently broad bases and pink flowers. A duplicate culture of 35304, the one from which the petiolate type was recovered was grown in 1936 and again in 1937 but no petioled types appeared in any of these populations. Owing to the fact that no petiolate types were encountered among other segregants from the original *N. triplex* selections this type was maintained irrespective of the absence of additional information pertaining to its origin.

Genetical Relationship between *N. triplex* Selections and Existing *N. tabacum* Types

With reference to varieties of *N. tabacum* grown for commercial purposes throughout the United States and Canada it may be said that morphological differences between these are slight. This condition is particularly surprising when it is realized that the number of varieties under cultivation is exceedingly large. To satisfy the requirements of the industry all varieties grown on this continent are listed under one or other of a number of major groups or types, such as flue-cured (Virginia or cigarette), dark air-cured, dark fire-cured, burley, cigar or pipe. Some of these types are subdivided still further and each of the types or subtypes contains many varieties which, with the exception of only a few instances, are not interchangeable. All these varieties possess lanceolate leaves which differ in relative length and width. All, in addition, possess pink coloured flowers with lobes distinctly separate, acute or pointed. Considerable difference, however, is observed in varietal vegetative development and relative maturity. There are other differences more difficult to detect which are of supreme economic importance, namely, flavour and aroma. Such characters constitute quality and quality is dependent upon colour, grain, texture, thickness, elasticity, etc., of the cured leaf. It would appear, that quality is influenced by a large number of factors which affect the physiological reaction of the plant and there is no information available respecting the involved factors responsible for quality. They are not sharply divided. Instead they appear to run through a range from very low to high. Such a condition can only be attributed to the presence of quantitative factors, the inheritance of which is exceedingly complicated in view of the fact that the effects of a number of factors or complex combinations are influenced by all the environmental conditions such as nutrition, temperature and light.

Since all varieties hybridize freely without segregation for flower colour or leaf shape, with the exception of slight modifications due undoubtedly to the presence of somewhat indistinct modifying factors, attention will be devoted to the genetical relationship between several *N. triplex* selections previously discussed, and two *N. tabacum* types. The first of these, 37072, (Calif. access. No. 06-25) was grown from seed obtained from Dr. R. E. Clausen in 1935. This variety, known as purpurea, has been selfed over a period of many years and on account of its established purity it has been used as the standard variety in many of the investigations pertaining to *N. tabacum* during the past twenty years, or more, at the University of California. It possesses a normal carmine coloured flower with a constricted leaf base. The second variety, referred to as 37288, is one of the most important cigar varieties on this continent. Since it, also, has been selfed for a number of years its purity should equal that of 37072. This variety possesses a normal pink flower and a lanceolate leaf base.

Case Number One

37081P_{11}	×	37072P_4
semi-broad leaf		constricted leaf
white flower		carmine flower

The F_1 of this population, 37409, possessed carmine flowers with semi-broad leaf bases. The leaf bases were not perfectly normal semi-broad. At the same time they were not strictly intermediate, but since they approximated the semi-broad type they were classified as such. The F_2 population, 38016, segregated for carmine and white flower colours, and semi-broad and constricted leaf bases. In this progeny there were 114 carmine flowers and 46 white.

From these figures it would appear that there is only one pair of factors involved. If it is assumed, as previously suggested, that carmine coloured flowers are conditioned by the expression of a factor Wh, in this instance, the white flower must be due to a pair of recessive factors, whwh. Such a condition accounts for the approximation of the simple monohybrid three-to-one ratio obtained in the F_2 population of this cross.

As for the leaf base forms of the F_2 population 38016 all were generally semi-broad, constricted or of the F_1 type. With the exception of the true constricted forms it was impossible to classify the others with reasonable accuracy. Most of the constricted forms, however, were fairly typical. On classification it was found that there were 48 constricted and 112 semi-broad or intermediate forms. According to work reported previously (Kelaney, 1925) a factor A, referred to as Br in this investigation is attributed to the broad leaf base character. If this is true the semi-broad leaf base may be due to the presence of the same factor as well as certain modifying factors. In such a case semi-broad leaf base would be represented by BrBrMM and when crossed with constricted, brbrmm, should give a variety of semi-broad, broad and constricted forms. The absence of normal broad forms in this population, however, eliminated such an hypothesis. It was felt, therefore, that this type, in reality a short winged petioled type, was due to the presence of a different factor, Wp, and responsible for the character here referred to as semi-broad. This, however, does not preclude the possibility of the presence of certain more or less obvious modifying factors.

The plants of the entire F_2 population, 38016, were classified for ultimate distribution as shown in table 6.

TABLE 6

Flower colour and leaf base segregation.

37081P_{11} × 37072P_4 F_2

Ratio	Phenotypes	Observed	Calculated
9	Carmine semi-broad	86	90
3	Carmine constricted	28	30
3	White semi-broad	32	30
1	White constricted	14	10

$\chi^2=2\cdot00$ $P=0\cdot5—0\cdot7$

37081P_{11}	×	37288P_3
semi-broad leaf white flower		lanceolate leaf pink flower

The first generation culture 37410 gave plants with carmine flowers, and leaf bases definitely intermediate in shape between lanceolate and semi-broad. The F_2 population, in which only 65 plants were classified, segregated for carmine, pink and white flower colours, there being 41 carmine, 11 pink and 13 white. These results are indicative of a two factor relationship and, also, are suggestive of the 9:3:4 dihybrid ratio referred to previously. As stated in the preceding pages pink flower colour may be represented by a WhWhpkpk genetic constitution. It must necessarily follow, therefore, that the white flower colour was whwhPkPk, which when crossed with pink, WhWhpkpk, produced a carmine, WhwhPkpk, F_1 population which in turn produced an F_2 population of carmine, pink and white segregants.

As for leaf base characters the F_2 population 38018 segregated for a number of forms which were difficult to classify. The major portion of this population, however, was sessile or partially so. The balance of the population consisted of 10 semi-broad parental types, 6 broad, 7 petiolate and 3 constricted. The other

forms exhibited various gradations of narrowly lanceolate to normal and broadly lanceolate shapes. The complexity of the segregation suggested the possibility of the presence of a three factor relationship. Lanceolate (Kelaney, 1925) in its simplest condition has the broad factor Br plus the petioled factor Pt. The Ptpt constitution gives a somewhat intermediate lanceolate type and Brbr a semi-broad type somewhat similar to the base of the semi-broad parent involved in this hybrid. It would appear, therefore, that the third factor, referred to above, is the same factor, Wp, which was considered in the discussion of the previous cross. This being the case the F_2 segregants should have consisted of eight phenotypes. Owing to the difficulty of distinguishing minor differences among the sessile forms complete classification of these was impossible. In any case it was possible to identify various classes of the types indicated below:—

WpBrPt WpBrpt WpbrPt wpBrPt	—Lanceolate forms—broad, narrow and normal
wpbrPt	—petiolate
wpBrpt	—broad
Wpbrpt	—semi-broad
wpbrpt	—constricted

Certain lanceolate forms included in this assemblage were of a long winged petiole type and these may have been of WpbrPt constitution. The other gradations would be represented by WpBrPt and WpBrpt, whereas the normal lanceolate would have been wpBrPt. In addition, it is possible that certain semi-broad types were of a heterozygous, Brbr, constitution since they would have been indistinguishable from the Wp type.

The plants of the entire F_2 population 38018 were classified for ultimate distribution as shown in table 7.

TABLE 7

Flower colour and leaf base segregation.

$37081P_{11}$ × 37288_3 F_2

Phenotypes	Observed
Carmine lanceolate forms	21
Pink lanceolate forms	9
White lanceolate forms	9
Carmine broad	4
Pink broad	1
White broad	1
Carmine petiolate	6
Pink petiolate	0
White petiolate	1
Carmine semi-broad	8
Pink semi-broad	1
White semi-broad	1
Carmine constricted	2
Pink constricted	0
White constricted	1

Case Number Two

$37087P_{24}$	×	$37072P_4$
broad leaf white flower		constricted leaf carmine flower

With respect to flower colour the F_1, 37412, as in the preceding cross involving $37072P_4$ as one of the parents were all carmine. The F_2 population, 38022, however, segregated for carmine, pink and white flowers instead of a 3:1 carmine

and white as expected. It was assumed, therefore, that the white parent 37087P_{24} was of a different genotype to that of the other white parent, 37081P_{11}, previously discussed. In this instance the white parent involved in the cross now under investigation must have been of a whwhpkpk genotype. The carmine parent being a standard type of known genetic constitution, WhWhPkPk, meant that the F_2 segregants of this cross could be accounted for only on the basis of a white flower possessing a different genetic constitution. Among the segregants of the F_2 population, 38022, were 92 carmine coloured flowers, 21 pink and 33 white. Three of the pink coloured flowers were almost white but, nevertheless, tinged with pink. The genetic constitution of the F_1 374112 was similar to that of the F_1 derived from 37081P_{11} $\times$ 37288P_3 and the F_2 phenotypes also was necessarily of the same order.

The leaf shape of the F_1 population plants was semi-broad and the F_2 segregated for 117 exhibiting evidence of the broad character and 29 definitely constricted. These results indicated a simple 3:1 monohybrid ratio in which a single pair of factors, Br-br, were involved.

The plants of the entire F_2 population, 38022, were classified for ultimate distribution as shown in table 8.

Table 8

Flower colour and leaf base segregation

37087P_{24} $\times$ 37072P_4 F_2

Ratio	Phenotypes	Observed	Calculated
27	Carmine broad	70	61·6
9	Pink broad	21	20·5
12	White broad	26	27·4
9	Carmine constricted	22	20·5
3	Pink constricted	0	6·8
4	White constricted	7	9•1

37087P_{24} $\times$ 37288P_3

broad leaf, white flower $\times$ lanceolate leaf, pink flower

The F_1 of the previous cross of white $\times$ pink involving 38081P_{11} and 37288P_3 was carmine and the F_2 segregated for carmine, pink and white flower colours. In this instance, the F_1 was pink and the F_2 segregated for pink and white flowers, 120 and 32 respectively. The difference between the behaviour of these two crosses is attributed to the differences in the genotypes of the two whites involved. As previously suggested the genetic constitution of pink may be represented by WhWhpkpk and white by whwhpkpk, in which case they differ only in a single pair of factors, hence, the monohybrid ratio as exhibited by the segregants of the F_2 population.

The leaf bases of the F_1 were more of the lanceolate order than broad. The F_2 population segregated for lanceolate forms and normal broads, 114 and 38 respectively. This relationship suggested a single factor difference. Since lanceolate is represented by a BrBrPtPt constitution the broad character must have been due to the presence of a pair of Br factors, BrBrptpt. Hence the 3:1 segregation in the F_2 of lanceolate and broad types.

The plants of the entire F_2 population 38024 were classified for ultimate distribution as shown in table 9.

TABLE 9

Flower colour and leaf base segregation

$37087P_{24}$ × $37072P_4$ F_2

Ratio	Phenotypes	Observed	Calculated
9	Lanceolate pink	89	85·5
3	Lanceolate white	25	28·5
3	Broad pink	31	28·5
1	Broad white	7	9·5

$\chi^2=1\cdot44$ $P=0\cdot5-0\cdot7$

Case Number Three

$37109P_{24}$	×	$37072P_4$
semi-broad leaf pink flower		constricted leaf carmine flower

The F_1 population was semi-broad and possessed carmine flowers. The F_2 gave 112 carmine to 34 pink. This is in substantial agreement with the results presented previously, based on the assumption of two pairs of factors, but differing only in one pair. In table 3 were reported ratios for flower colour analogous to those encountered here. It may be assumed that it is possible to represent plant $37109P_{24}$ by a WhWhpkpk genetic constitution, pink, and 37072 by WhWhPkPk, carmine.

The leaf base types segregated for semi-broad and constricted, 113 and 33 respectively which suggested a single factor difference and a 3:1 ratio. The behaviour in this instance was comparable to that which was discussed under Case Number One in which a similar cross was reported.

The plants of the entire F_2 population, 38054, were classified for ultimate distribution as shown in table 10.

TABLE 10

Flower colour and leaf base segregation

$37109P_{24}$ × $37072P_4$ F_2

Ratio	Phenotypes	Observed	Calculated
9	Semi-broad carmine	87	82·1
3	Semi-broad pink	26	27·4
3	Constricted carmine	25	27·4
1	Constricted pink	8	9·1

$\chi^2=0\cdot71$ $P=0\cdot8-0\cdot9$

$37109P_{24}$	×	$372883P_3$
semi-broad leaf pink flower		lanceolate leaf pink flower

With respect to flower colour all plants in both F_1, 37431, and F_2, 38056, populations were pink. This condition demonstrated the existence of similar factors responsible for flower colour in both parents.

As for leaf shape the F_1 approximated the lanceolate type and the F_2 segregated for a multitude of types which were predominantly of a variety of lanceolate forms, a few of which resembled the normal broad leaf base. In all there were 72 lanceolate forms, 24 which resembled the long winged petiolate type, 28 short winged petiolate or semi-broad, 9 definitely petiolate, and 5 constricted. Such a class of phenotypes suggested that the semi-broad character displayed by the parent, $37109P_{24}$, was not genetically semi-broad but, instead, winged petiole, short, as described for $37081P_{11}$ in the discussion of Case Number One, $37081P_{11} \times 37288P_3$. Since this population was considerably larger than the comparable one previously discussed it should be more representative of the true situation. Hence, the various phenotypic classes are presented below showing the number of individuals classified in each group:

Calculated		*Actual*
27	WpBrPt.	96 Lanceolate forms, broad, normal and narrow.
9	wpBrPt.	
9	WpbrPt.	
9	WpBrpt.	
3	Wpbrpt.	30 Semi-broad (short-winged petiole)
3	wpBrPt.	6 Broad normal
3	wpbrPt.	9 Petiolate
1	wpbrpt.	5 Constricted

By way of explanation it must be remembered that it is possible to have individual semi-broad types due to a set up such as Wpbrpt, as well as the expression of the heterozygous broad Brbr, genotype. This condition, and probably others, may account for the relatively large number of individuals in the Wpbrpt class. In addition it was not known which of the character expressions belonged to the WpBrpt class. It is not impossible that some of the 30 individuals classified as semi-broad should have been more correctly placed in this group. As for the narrowly lanceolate forms which approached a long winged petiolate character it was felt that they may have been assigned to the WpbrPt class until further evidence is available pertaining to the genetic constitution of this particular character.

Case Number Four

$37090P_5$	$\times$	$37072P_4$
broad leaf base		constricted leaf base
pink flower		carmine flower

All plants in the F_1 population, $37414P_3$ were decidedly sessile and could not be classified as anything but broad. All possessed carmine flower colours. The F_2 population, 38030, gave 110 carmine and 32 pink in support of the claims previously presented respecting the factor relationship existing between carmine and pink flower colours. Among the various segregants for leaf base types were 107 broad and 35 constricted. These results confirm a single factor relationship and that the broad type may be an expression of dominant factors BrBr, and the constricted leaf base the expression of a pair of recessive factors brbr.

The plants of the entire F_2 population, 38030, were classified for ultimate distribution as shown in table 11.

TABLE 11

Flower colour and leaf base segregation

$37090P_5$ × $37072P_4$ F_2

Ratio	Phenotypes	Observed	Calculated
9	Carmine broad	76	79·8
3	Carmine constricted	34	26·6
3	Pink broad	31	26·6
1	Pink constricted	1	8·9

$\chi^2 = 9 \cdot 99$ $P = 0 \cdot 01 - 0 \cdot 02$

These results suggest linkage between flower colour and leaf shape as indicated in tables 5 and 8.

$37090P_5$	×	$37288P_3$
broad leaf base		lanceolate leaf base
pink flower		pink flower

The flower colour of the first generation plants, 37416, were all pink and the leaf bases were lanceolate. The F_2 population, 38032, segregated for leaf base types only, lanceolate and broad, 112 to 30, respectively. Although the theoretically expected values were closely approximated it must not be assumed that the differences were distinct. The various segregants could not have been definitely classified from field observations alone. The assistance of the line drawings was essential. It would appear from the analysis that both types, broad and lanceolate may be explained on monohybrid assumption. Since the expression of the broad character behaves as a dominant condition over constricted it is possible to account for the relationship existing between lanceolate and broad by resort to a second pair of factors such as suggested by Kelaney (1925) and others. The lanceolate type may be represented by BrBrPtPt and the broad base type by BrBrptpt. The F_1, as observed, should be lanceolate, BrbrPtpt and the F_2 behaved according to the expected 3 lanceolate to 1 broad.

Case Number Five

$37112P_8$	×	$37072P_4$
long wing petiolate		constricted
carmine		carmine

The plants in the F_1 progeny, 37432, were very much of the winged petiolate type and all possessed carmine coloured flowers. The F_2 segregants, 38060, consisted of all carmine flowers. In this connection the results are in agreement, with respect to genetic relationships, with the observations previously made on carmine flower colour. As for leaf base shapes the segregants consisted of 113 winged petiolate to 31 constricted. In view of results discussed later pertaining to crosses involving a typical petiolate base type it does not seem reasonable to assume that the long winged petiolate form and the petiolate form are expressions conditioned by the same factor or factors. Neither must it be assumed to be genetically equivalent to the semi-broad or short winged petiole

type, previously discussed, since there is a conspicuous morphological difference between them. To determine the factor relationships of the long winged petiolate type more definitely this type should have been crossed with both the normal petiolate and semi-broad types and a study made of the F_2 segregants. Unfortunately inter-crossing of the *N. triplex* selections was not conducted. Interpretations of genetic constitutions, therefore, must be confined to the results obtained from the present investigation. In this instance the segregants of the F_2 population can be accounted for if it is assumed that the long winged petiolate character is attributed to a factor which may be referred to as Pw. In such an instance the cross involving long winged petiolate may be expressed as follows:

PwPw (winged) × pwpw (constricted)

F_1 Pwpw (intermediate)

F_2 1 PwPw : 2 Pwpw : 1 pwpw
winged constricted

Such a representation could be accepted as an explanation of the cross under discussion.

$37112P_8$ × $37288P_3$

long winged petiolate carmine — lanceolate pink

In this instance there was no segregation for flower colour in the F_1 population. The F_2 population, 38062, consisted of 64 carmine and 19 pink which was in agreement with the explanations offered previously for similar crosses. As for leaf shapes a variety of forms were observed among the segregants although they were predominantly lanceolate. Other types included long winged petiolate shapes as well as a lesser number of broad, petiolate and constricted ones. The phenotypic classes observed here were similar to the ones encountered when the semi-broad, or short winged petiolate type was crossed with lanceolate, $37288P_3$. It would therefore appear that the factor responsible for the long winged petiole character may be identical with the factor attributed to the expression of the semi-broad or short winged petiole type but the difference between the two characters is so marked that such an assumption would not be warranted until the genetic relationships of these types is studied as a result of further investigation. The various phenotypic classes are presented in table 12 showing the number of individuals classified in each group:

TABLE 12

Flower colour and leaf base segregation

$37112P_8$ × $37288P_3$ F_2

Calculated	Actual	Carmine	Pink
Ratio and classes—			
27 PwBrPt	Lanceolate form	37	16
9 pwBrPt	Lanceolate form	37	16
9 PwBrPt	Lanceolate form	37	16
9 PwbrPt	Lanceolate form	37	16
3 Pwbrpt	Long winged petiolate	18	0
3 pwBrpt	Broad	2	3
3 pwbrPt	Petiolate	5	0
1 pwbrpt	Constricted	2	0

As previously suggested certain long-winged petiolate plants may have been narrowly lanceolate forms of the PwbrPt type instead of the true Pwbrpt type, hence the predominance of long-winged petiolate types as classified above.

Case Number Six

$37094P_{46}$	$\times$	$37072P_4$
winged petiolate base carmine white throat pale filaments		constricted carmine

The F_1 population, 37418, were winged petiolate but the petiole was somewhat shorter in length than the winged petiolate parent. All the plants were normal carmine in colour. The F_2 progeny, 38036, gave 112 carmine to 21 carmine white throat and pale filaments. These results approximate a 3:1 relationship and it is suggested that the character expressed is due to a single factor difference.

The leaf base forms yielded 96 winged petiolate type to 37 constricted. These results are comparable to those obtained for Case Number Five above. $37112P_8$ winged petiolate $\times$ $37072P_4$ constricted. The plants for the entire F_2 population, 38036, were classified for ultimate distribution as shown in table 13.

TABLE 13

Flower colour and leaf base segregation

$37094P_{46}$ $\times$ $37072P_4$ F_2

Ratio	Phenotypes	Observed	Calculated
9	Carmine long winged	80	74·8
3	White throat long winged	16	24·9
3	Carmine constricted	32	24·9
1	White throat constricted	5	8·3

$\chi^2=6\cdot85$ $P=0\cdot05-0\cdot10$

$37094P_{46}$	$\times$	$37288P_3$
winged petiolate base carmine white throat pale filaments		lanceolate pink

In culture number 37418, the F_1 population derived from the above cross, the flowers appeared to be normal carmine in colour and the leaf bases almost lanceolate in shape. The F_2 population, 38038, segregated for normal carmine, carmine white throat and pale filaments, and pink flower colours, 67, 14 and 38, respectively. Since the white throat and pale filament character seems to behave as a recessive in the first generation it may be attributed to a factor W. On this assumption a 9:3:3:1 ratio would have been expected, as follows:—

9 PkPkWW	:	3 PkPkww	:	3 pkpkWW	:	1 pkpkww
carmine		carmine white throat pale filaments		pink		pink

Since no difference was noted between the various pink genotypes the ratio becomes a 9:3:4.

The segregation for leaf base shapes in the F_2 compared favourably with the results reported above when long winged petiole was crossed with lanceolate. Among the segregants were 60 lanceolate, 47 winged, 7 petiolate, 4 broad and 1 constricted.

The plants of the entire F_2 population, 38038, were classified for ultimate distribution as shown in table 14.

TABLE 14

Flower colour and leaf base segregation

$37094P_{46}$ × $37288P_3$ F_2

Classes	Observed
Carmine lanceolate	27
White throat lanceolate	2
Pink lanceolate	31
Carmine long winged petiolate	34
White throat, long winged petiolate	11
Pink long winged petiolate	2
Carmine broad	0
White throat broad	0
Pink broad	4
Carmine petiolate	5
White throat petiolate	1
Pink petiolate	1
Carmine constricted	1
White throat constricted	0
Pink constricted	0

Case Number Seven

$37098P_{14}$	×	$37072P_4$
constricted leaf carmine white throat pale filaments		constricted carmine

The F_1, 37421, were all constricted with respect to leaf base and all possessed normal carmine flower colours. The F_2 progeny, 38042, was not large owing to insect injury in the garden. A number of plants were destroyed in the field shortly after transplanting. Out of a total of 89 plants, 71 were normal carmine and 18 were carmine with white throats and pale filaments. These results also suggest a monohybrid 3:1 relationship. In addition all the plants in this population possessed constricted leaf bases which indicated a common genetic factorial constitution.

$37098P_{14}$	×	$37288P_3$
constricted leaf carmine white throat pale filaments		lanceolate pink

The F_1 population, 37422, approximated the lanceolate leaf type and all were normal carmine in flower colour. The F_2 population, 38044, yielded 61 plants with normal carmine colour, 5 carmine with white throats and pale filaments and 31 pink. As for leaf bases there were 67 lanceolate, 17 petiolate, 7 broad and 6 constricted. Although there seemed to be insufficient broad forms these figures, however, approximated a 9:3:3:1 ratio. The only difference between these results and those obtained by Kelaney (1925) exists in the fact that the largest number of plants in either of the categories is classified as lanceolate instead of petiolate. The heterozygous forms were not strictly petiolate. Instead they approximated the lanceolate type.

This population 38044 was somewhat reduced in size owing to loss of seedlings from insect injury after transplanting, but the remaining portion was classified for ultimate distribution as shown in table 15.

TABLE 15

Flower colour and leaf base segregation

$37098P_{14}$ × $37288P_3$ F_2

Classes	*Observed*
Carmine lanceolate	38
White throat lanceolate	2
Pink lanceolate	27
Carmine petiolate	14
White throat petiolate	2
Pink petiolate	1
Carmine broad	4
White throat broad	1
Pink broad	2
Carmine constricted	5
White throat constricted	0
Pink constricted	1

Case Number Eight

$37137P_3$	×	$37072P_4$
petiolate		constricted
pink		carmine

As previously stated, the petiolate type may not have been a legitimate selection from the original *N. triplex* population 35304, which was the progeny derived from P_{21} a selection from culture number 35157. However, the type was obtained and it continued to yield petiolate forms. When crossed with constricted it gave an F_1, 37446, with petiolate leaves. The F_2, 38076, segregated for petiolate and constricted 132 and 32. The difference between the segregants was quite marked and only little difficulty was encountered in classifying them. The results suggested a 3:1 ratio which would indicate a single factor difference in genetic constitution. As shown previously, the constricted leaf base brbr is recessive to broad BrBr. It also appears that it is recessive to petiolate. It is assumed therefore that petiolate must be attributed to the factors PtPt and that constricted is attributed to ptpt, ptptbrbr or brbr.

The segregation for flower colour compared favourably with the results reported above in which pink and carmine crosses were involved. The F_1 population, 37446, were all carmine and the F_2, 38076, produced 117 carmine and 47 pink in substantial agreement with factor relationship previously stated.

All the plants in the F_2 population, 38076, were classified for ultimate distribution as shown in table 16.

TABLE 16

Flower colour and leaf base segregation

$37137P_3$ × $37072P_4$ F_2

Ratio	Phenotypes	Observed	Calculated
9	Carmine petiolate	89	92·3
3	Carmine constricted	28	30·7
3	Pink petiolate	43	30·7
1	Pink constricted	4	10·2

$\chi^2=8\cdot82$ $P=0\cdot02-0\cdot05$

$37137P_3$	$\times$	$37288P_3$
petiolate		lanceolate
pink		pink

The F_1 or F_2 progenies 37447, and 38078 showed no segregation for flower colour. The F_1 37447 and a duplicate culture 38077 possessed leaf base shapes which could not be designated petiolate if the "petiolate" expression is to be confined to the petiolate type of leaf base possessed by the parent $37137P_3$. If the term is to be used indiscriminately for various forms of petiolate leaves it may also be extended to apply to lanceolate leaf bases as well. The F_1 of this population were not petiolate as anticipated (Kelaney 1925). Instead they were lanceolate in nature characterized by a fairly broad wing, definitely rugulose, and decurrent. The F_2 population 38078 provided some difficulty for purposes of classification. No broad, semi-broad, winged petiolate, constricted, etc., types were encountered. All seemed to belong to the normal lanceolate, petiolate or F_1 group which was essentially lanceolated. An analysis showed that there were only 33 definitely petiolate forms and 119 which could not escape other designation but lanceolate if the standards throughout this investigation were to be maintained.

Summary and Discussion

Although the studies reported in this investigation deal primarily with the genetic relationships involved in certain flower colour and leaf base characters, an effort was made to determine the hereditary aspects of other features such as number and size of leaves, height of plant, and relative maturity. Comparable investigations of this kind have been conducted in the past mainly by Hayes (1913) and East (1916 and 1928). Since the results from the present study, with respect to these characters, are analogous to those previously reported it was felt that it was unnecessary to publish the author's data at this time. It may be said, however, that the differences could not be explained on a simple factorial basis. Instead the results demonstrated the complexity of the genetic differences in the parental strains and the validity of the multiple factor hypothesis as a description of the heredity of quantitative characters.

With reference to flower colour investigations which involved white, pink, normal carmine and one other carmine, which possessed an almost colourless tube or infundibulum as well as pale filaments, it was shown that the characters were inherited on a comparatively simple Mendelian basis. Carmine, pink and white flower colours appear to be controlled by two pairs of factors WhWh and PkPk in which the symbols Wh and wh are used to denote coloured vs. white and Pkpk for carmine vs. pink. Thus carmine flower colour may be WhWhPkPk; pink WhWhpkpk; and white whwhPkPk or whwhpkpk. Carmine WhWhPkPk $\times$ white whwhPkPk segregated in the F_2 for 3 carmine: 1 white and, in the second instance, carmine WhWhPkPk $\times$ white whwhpkpk segregated in the F_2 for 9 carmine: 3 pink: 4 white. Pink, WhWhpkpk when crossed with the first white, whwhPkPk gave a carmine F_1 and an F_2 population of 9 carmine: 3 pink: 4 white and when crossed with the second white gave a pink F_1 and a 3 pink: 1 white in the F_2 population. When carmine and pink were intercrossed, the F_2 segregated for 3 carmine: 1 pink. These results, in addition to being in general agreement with Clausen (1932), also support the hypothesis that the Wh and Pk factors are carried on two separate chromosomes, designated C and P, respectively. By virtue of the origin of the various strains or selections upon which this investigation is based it is not impossible to have had certain chromosomes, or at least certain genic elements contributed by either the *sylvestris* or *tomentosiformis* parents. If the factors for colour vs. white and

carmine vs. pink are carried on different chromosomes it was not surprising, therefore, to witness the effects of independent assortment as reported in this investigation.

The character displayed by certain carmine types and described as carmine with white throat and pale filaments behaved as a simple monohybrid recessive when crossed with normal carmine. The F_2 population segregated for 3 normal carmine: 1 carmine and white throat and pale filaments. When this type was crossed with normal pink only carmine, carmine white throat and pale filaments, and pink were observed in the F_2 population in a significant 9: 3: 4 ratio. If another factor W is introduced, the recessive condition of which in the presence of Pk, carmine, produces white throat and pale filaments it must be assumed that W itself fails to alter the expression of normal flower colour in the presence of either the factors Pk or pk. The double recessive pkpkww type was not distinguishable from the normal pink.

In addition to the observations just reported there were other flower colour features encountered in certain segregating populations which were more perplexing in their nature. Various shades of carmine, pink and even white were recognized. Certain carmine coloured flowers approximated a deep red, and pink appeared to vary in intensity. As for white, three instances were recorded during the past summer when dull cream coloured flowers appeared in segregating populations which could not be accounted for on a factorial basis in the light of available information. It would appear that the presence of modifying factors exerts a significant influence on the expression of such characters. Further investigation is warranted, and time is required before contentions of this kind are definitely determined. As for other characters, such as variegated forms, a cytological analysis appears to be desirable.

With respect to the genetics of leaf shapes the most outstanding contribution in the past was presented by Setchell, Goodspeed, and Clausen (1922). The results of their work were fully reviewed by Kelaney (1925). The writer's results agree in general with those of Kelaney (1925). There are, however, certain instances of disagreement due, in part, to:

(1) A failure on the part of the latter to define adequately his types and to adhere strictly to established standards. The petiole, for instance, of the petioled type is described as being variable in length and wing development. Since a definite petiole length without significant variability can be established (See Plate IV) it is the writer's opinion that at least two factors are involved. In addition, if variability in wing development is accepted one encounters extreme difficulty in classifying or identifying all gradations between lanceolate and strictly petiolate forms.

(2) A failure also to recognize additional leaf base shapes. (See plate III and V fig. 1). Although modified semi-broad or winged petiolate forms were encountered in F_2 segregating populations of lanceolate $\times$ constricted it does not necessarily follow that the shape is conditioned only by heterozygous factors and is incapable of establishment. These types were established during the course of the present investigation from *triplex* selections. Similar types were observed in a number of segregating populations and their presence could not be explained in every instance on the basis of their predetermined genetic constitution. This condition provides considerable evidence in support of the suggestion advanced by Setchell, Goodspeed, and Clausen (1922) that similar morphological expression may differ in genetic constitution. In fact this phenomenon creates a difficulty of some magnitude in classifying segregating populations since leaf shapes, probably limited in number, frequently occur that cannot be explained on a factorial basis in agreement with the known constitution of similar shapes and with the known constitution of the parents.

The results of investigations here reported are, as previously stated, in general agreement with those of Kelaney (1925). Broad leaf base is attributed to the presence of Br factors. Petiolate bases are attributed to Pt factors. The presence of both pairs of factors, PtPtBrBr, is responsible for the lanceolate leaf base and the recessive condition, ptptbrbr, produces the constricted leaf base. According to Kelaney (1925) heterozygous factors PtptBrbr gave short petioled leaf bases, and PtPtBrbr gave petioled leaf bases. In the work here reported genetic constitutions of this nature gave leaf bases which approximated the lanceolate forms.

In addition winged petiolate was attributed to the presence of a factor designated as Pw. It is possible that this factor is quite independent of the factor Wp responsible for the semi-broad or short winged petiolate type. Yet, at the same time it is possible, although there is no evidence to substantiate the claim, that they may be members of an allelomorphic series differing only in a quantitative way. Further complexity in the classification of leaf shapes was experienced in dealing with the semi-broad or short winged petiole type. This type was attributed to the presence of a Wp factor but certain of these, as classified, may have been of a Brbr constitution. In addition, the long winged petiolate type, Pw, may have been morphologically similar to WpPt and PwPt.

Although time did not permit a study of linkage values specific evidence of this phenomenon was observed in the study of F_2 populations. From tables 5 and 8, for instance, it will be noticed that no pink constricted plants appeared in the populations under investigation. These results are indicative of a very close linkage relationship between Br and Pk factors as previously reported by Kelaney (1925) and Clausen (1932).

In conclusion it may be said that during the course of this investigation a number of additional types of leaf bases have been established showing minutely graded differences between petiolate and lanceolate forms and short petiolate and broad forms. Although the presence of multiple allelomorphs controlling graded differences in leaf shapes has not been established it seems that such a hypothesis may be worthy of some consideration. A series of factors controlling petiole length and wing development may be regarded as differing from one another on a quantitative basis. Results of several investigations of this kind have been reported in the past. Agol (1931) pointed out that a series of factors controlled wing shape in *Drosophila* and Tjebbes (1931) reported on triple allelomorphic factors showing a graded difference in the expression of characters affecting pod and foliage colour in *Phaseolus*. Whether there are multiple allelomorphic series of factors in *N. tabacum* exhibiting quantitative differences in expression can only be determined by future investigations of an intensive nature.

It would appear from the results presented in this paper that the *N. triplex-purpurea* selections, referred to throughout as *N. triplex* selections, do not differ extensively from previously existing *N. tabacum* types. No evidence was found which suggested that there was any greater genetic differences between the *triplex* selections and *tabacum* types than the suspected differences between the two types designated as 37072 and 37288. Although the characters which have been investigated were essentially morphological in nature it is not assumed that only morphological differences between the types were to be expected. That physiological differences of a quantitative nature exist is by no means denied.

Conclusions

1. Two pairs of factors account for the relationship existing between carmine, pink and white flower colours. These segregated as follows:

9 WhWhPkPk	carmine
3 WhWhpkpk	pink
3 whwhPkPk	white
1 whwhpkpk	white

2. The white throat and pale filaments of carmine-coloured flowers is attributed to the recessive condition of a third factor, ww.

3. Lanceolate, broad, petiolate and constricted leaf base types are attributed to the following factors:

BrBrPtPt	lanceolate
BrBrptpt	broad
brbrPtPt	petiolate
brbrptpt	constricted

4. A third factor, Wp is necessary to account for semi-broad or short-winged petiolate type.

WpWpbrbrptpt semi-broad or short winged petiole

5. The long-winged petiolate type which is morphologically distinct from the semi-broad or short-winged petiolate type is attributed to another factor Pw.

PwPwbrbrptpt long-winged petiole

6. The factors, Br-br, for broad vs. constricted leaf base, and Pk-pk, for carmine vs. pink, are very closely linked.

7. The assortment of characters derived through progeny selection from a triple fertile hybrid suggests a valuable means of introducing new characters in plants of polyploid origin.

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